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SOME EFFECTS OF MULTIPLE SUB-LETHAL THERMAL SHOCKS

ON SCHOOLING BEHAVIOR AND PREDATION SUSCEPTIBILITY

OF FATHEAD MINNOWS (Pimephales promelas) ACCLIMATED AT 18° C.

schoole predation

bу

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# TABLE OF CONTENTS

	Acknowledgements	
	Introduction	
3.	Materials and Methods	5
4.	Results and Discussion	13
5.	Summary	27
6.	Literature Cited	29
7.	Vita	31
Q	Abstract	

#### INTRODUCTION

Increasing demand for energy has been accompanied by a growing stress on the environment. Electrical energy has become the most important form of energy in the United States with power needs doubling approximately every 10 years (Levin et al. 1972). Many conventional methods of electrical energy production are finite in the amount of energy they can produce. Hydroelectric facilities are being increasingly limited physically by possible site locations and legally by recent environmental protection legislation. Fossil fuel supplies are rapidly dwindling. Use of such inexhaustable potential sources as the wind, tides, and solar energy have not yet become technologically or economically feasible on a large scale.

At present, steam-electric power plants supply the majority of electric power in the United States and are expected to supply over 90% of the requirements by the year 2020 (Levin et al. 1972). These plants require tremendous amounts of water for condenser cooling. According to the 1968 report of the Water Resource Council, cooling water comprised about 33% of the withdrawal from this country's water resources in 1965. By 1980 cooling water will account for about 44% of the withdrawal, and by the year 2020, 67% of the total (Levin et al. 1972). Recycling of cooling water is not and probably will not be economically feasible in the near future.

At present, the only economically acceptable solution to the problem of thermal waste disposal is to establish biologically and economically justifiable limits on the rates and times that heated

water can be released into our water systems. Hagen (1972) notes that, though several useful guidelines have been established through study of a few key species of fish, each new source of pollution will require modifications in effluent guidelines based on the dynamics of the ecosystem and the particular species involved. Cairns (1972) states that one must estimate how much the "natural" temperature of a particular body of water may be altered without adverse effects upon the aquatic community. He also notes that in order to maintain most aquatic communities, a seasonal cycle must be retained, and changes in temperature must be gradual. This conclusion may also be applied to daily cycles. Tolerance limits on the rate of temperature change depends on the type of aquatic community being considered. When fish are exposed to rapid temperature change with little or no time for acclimation, thermal shock commonly occurs, the seriousness of the shock depending largely on the degree of temperature difference and also on the species of fish involved (Hagen 1972).

Many organisms have been studied with respect to lethal levels of thermal stress, but little is known about sublethal effects. Coutant (1970) suggests that behavior, performance (i.e. feeding efficiency), metabolism, food chain relationships, community structure, and natural selection may be affected by thermal changes. Hagen (1972) also notes that sublethal thermal stresses will affect feeding and growth, with resulting changes in body weight and stamina leading to increased vulnerability to parasites and disease.

Alabaster (1963) found that fish can be stressed by very small temperature changes. Survival of fish under such stress is based upon

the resulting physiological and behavioral changes and, as Hagen (1972) notes, the ability of the fish to compensate for the stress. Olla and Studholm (1971) found that temperature stress in bluefish (Pomatomus saltatrix L.) caused changes in swimming rate, schooling behavior, and daily rhythmicity. Changes in metabolic rate (Brown 1957, Bennett 1971, Becker 1971, Hagen 1972) and activity (Becker 1971) and disturbance of biochemical homeostatic mechanisms (Wedemeyer 1973), resulting from changes in water temperature, may cause detrimental behavioral changes. As Hagen (1972) notes, it is obvious that any temperature change in the aquatic environment will have an ultimate effect on any fish population present.

A predator-prey relationship depends on many aspects of behavior of both the predator and the prey. Any change in the behavior of either may affect the entire relationship. Sublethal temperature shock may upset protective behavior patterns of schooling minnows which comprise a major part of the food source of many game and food fish species.

Baerends (1971) considered schooling a protection against predators because single fish often join a school when frightened. Eibl-Eibesfeldt (1962) noted that a predator's effectiveness in catching a minnow decreased when the minnow joined a school. Thus, susceptability to predation may be determined in part by the schooling behavior of the prey.

Most organisms follow rhythmic cycles in their movements and feeding behavior. Snow (1971) showed that peaks of angler c/f (catch per unit of effort) for largemouth bass (Micropterus salmoides) occurred in early morning and again in the evening. These peaks of c/f may indicate

higher feeding rates of the bass during those periods.

In the case of the predator-prey relationship, rhythmic feeding of the predator may force the prey organisms into similar rhythms of avoidance behavior. Many small fish will seek cover in shallow water when pursued by predators. In deep water, the forage fish are often forced to the surface while attempting to escape predation.

In lakes and reservoirs where heated water is discharged, the hot water tends to remain at the surface (North and Adams 1969). As small fish are pursued by predators, their movement into shallows or to the surface could force them into a potentially thermal stressing situation. Thus, repeated stressing of prey could result from the periodicity of predator feeding.

The objective of this study was to evaluate possible changes in the susceptibility of a prey species to predation after periodic thermal stress and to evaluate changes in behavior, or signal responses (Hagen 1972), resulting from these stresses.

#### MATERIALS AND METHODS

All tests were performed under a constant 12 hr photoperiod and 20C air temperature. The 20C air temperature maintained a water temperature of 18C in all tanks.

I constructed four plywood testing tanks measuring 112 x 47 x 47 cm (inside). Two observation tanks had one side and one end constructed of clear plexiglass. These observation tanks were set up as mirror images of one another and were oriented so that the interiors of both tanks were visible at the same time to one observer (Fig. 1). Air stones were suspended approximately 12 cm above the bottoms of the tanks. Non-functional water hoses were present in the control tank at the same locations as the intake and outflow hoses in the stressing tank.

All fish were given a prophylactic treatment with malachite green, methylene blue, Furacin, and salt prior to beginning the experiments. Fathead minnows (<u>Pimephales promelas</u>), the prey, were kept in a holding tank consisting of a Living Stream (made by Frigid Units, Inc., Toledo, Ohio) with cooling unit and flow panel removed. Largemouth bass (<u>Micropterus salmoides</u>), the predators, were kept in the two all-plywood tanks which were used for predation tests. Fish were acclimated to the test environment for at least 14 days before the experiments were begun.

Water temperature in the stressing tank was manipulated by an overflow water exchange system. The system consisted of two 190 liter reserviors and a pump controlled in part by a thermoregulator in the stressing tank (Fig. 1). To raise the water temperature in the stressing

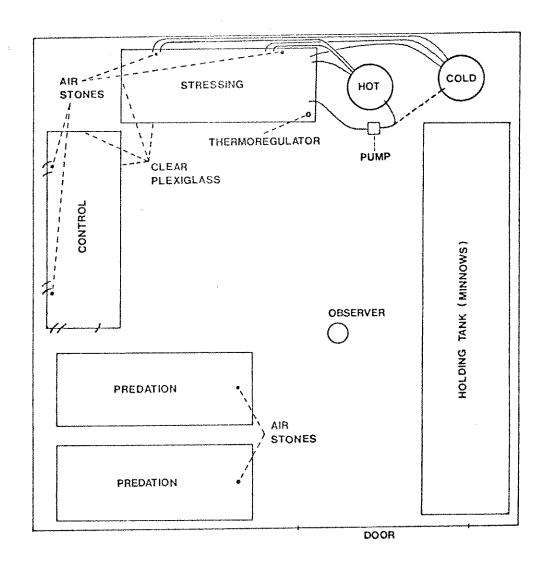


Figure 1. Experimental apparatus enclosed in an environmental control room.

tank to the selected level, cool water was pumped out of the stressing tank into the hot water reservior. The inflow caused hot water to overflow back into the stressing tank. When the temperature in the stressing tank reached the desired level, the pump automatically shut off. The thermoregulator maintained the selected temperature in the tank by activating the pump when the temperature began to fall. To return the temperature in the stressing tank to the pre-stress level, the pump outflow was directed into the cold reservoir. The pump was then operated until the cold water overflow had returned the temperature in the stressing tank to the acclimation level.

Two groups of equal numbers of minnows were randomly selected from the holding tank for each test. These two groups, control and treatment, were marked by removing pelvic fins on opposite sides of the body. Both groups were acclimated to the observation tanks for three days, then the fish in the stressing tank were subjected to stress periods of high temperature twice daily for six days and once on the morning of the seventh day. For each stress period the temperature was raised from the acclimation temperature (18C) to 32C, a previously determined maximum sublethal temperature, held for 1 hr 25 min, then returned to 18C. The change between 18C and 32C took about 20 min (Fig. 2). At 32C, the "Critical Thermal Maxima" (Hagen 1972) with an 18C acclimation level, the fish exhibited erratic swimming, loss of buoyancy and equilibrium control, stupor, and convulsions. Above this temperature many fish died. I considered this temperature behaviorally lethal for fish acclimated to 18C because they would be unable to avoid predation in such a state.

During the six days of stressing in each test, observations on various aspects of behavior were made on the control and stressed fish at 15 fixed times each day (Table 1). These observations included activity level (as judged by swimming and ventilation rate), school size and dispersal, and the amount of straying from the school when a school had been formed. Observations for periods of temperature change are averages for those periods.

Activity was subjectively estimated as low (L), medium (M), high (H), of very high (VH), low and very high being the extremes of activity levels. These estimations were given weighted values (L=1, M=2, H=3, VH=4) for statistical analysis.

School size was compared using a 10 x 10 cm grid marked on the inside rear walls of the tanks. Size was recorded as either the length or height of the school in squares. Only one dimension was recorded since the school generally maintained a depth dimension of one to two squares. Group dispersion was recorded as 9.5 when fish were dispersed over 1/2 of the tank or 15 when dispersed over all of the tank. These values were computed by summing length and height (in squares) of the area occupied by the school. I used these values instead of the total areas covered by the group because it allowed rapid occular estimates with a differentiation between schooled and dispersed groups.

Strays from the school were recorded as zero, one, few, or many because of difficulty in counting all strays in both tanks at the same time. The observations were given weighted values (zero=0, one=1, few=2, many=3) for analysis.

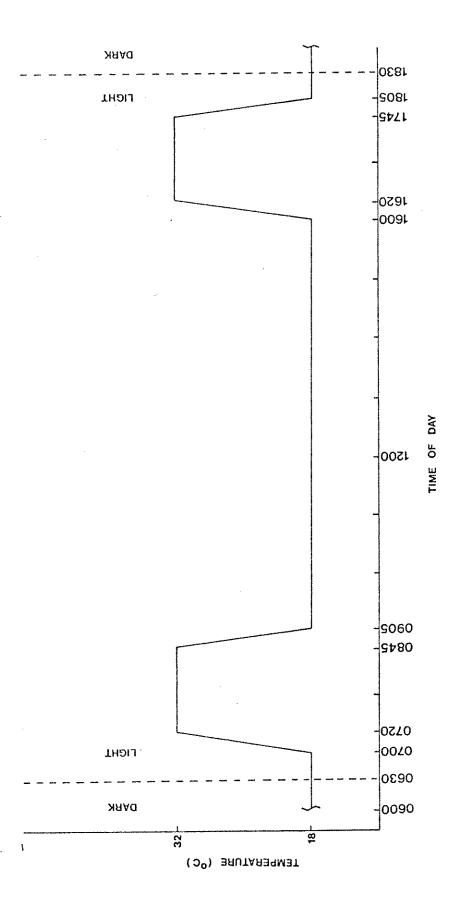


Figure 2. Daily temperature stressing schedule in treatment tank.

Table 1. Daily observation periods.

OBSERVATION PERIOD	TIME
1	0655
2	0700-0720
3	0725
4	0800
5	0840
6	0845-0905
7	0910
8	1230
9	1555
10	1600-1620
11	1625
12	1700
13	1740
14	1745-1805
15	1810

One preliminary and four standardized tests were completed. In the preliminary test, only general behavior observations were made. Attempts to incite fright reaction (school formation accompanied by high swimming and ventilation rates) with artificial bass were ineffective. Rapid movement by the observer while in the room proved to be an effective means of producing a fright reaction by which test and control groups could be compared. Though no measurements were made on these reactions, observations were noted and are discussed later.

In the preliminary test I also attempted to use "Viewing Ports" as suggested by Foster et al. (1969), modified by providing a cloth cover over the slit and observer. Such a cover blocked out light entering through the slit and theoretically made the observer invisible to the fish. When the fronts of the tanks were covered, the tops of the tanks were uncovered to allow light entry. With the tops of the tanks uncovered fish maintained high ventilation rates and any disturbance would incite rapid swimming and tight school formation. To avoid this complication in the remaining tests, I removed the viewports and covered the tops of the tanks. Observations were made from the side of the room farthest removed from the tanks. These standardized methods and observations were used for the four remaining tests.

Predation tests followed each week of temperature stressing and behavior observation. Following the morning stressing period of the seventh day, equal numbers of fish were removed from the observation tanks. Groups composed of 50% stressed and 50% control fish were placed in the predation tanks, one in each tank. When the bass had consumed

approximately 50% of the minnows in a tank or after 2 hours of test time, whichever occurred first, the remaining minnows were removed from the tank. The number of surviving stressed and control fish were recorded. Experiments generally followed the flow diagram given by Coutant (1973) for testing effects of thermal shock on vulnerability of juvenile salmonids to predation.

# RESULTS AND DISCUSSION

# Predation Susceptibility

Behavior changes of predators caused several problems throughout the experiment. Dominance, territorial conflicts, and decline in feeding rate necessitated the periodic removal and replacement of bass during the project. After test IV (Table 2) it became necessary to replace the original bass with younger bass presumably having less intense dominance behavior and higher feeding rates. The younger bass did initially have higher feeding rates, but hierarchies soon developed and feeding rates declined. Changes in feeding rates and number of predators necessitated varying the number of prey used in each predation test, making it necessary to modify the data analysis.

Results of the predation tests (Table 2) were analyzed using a modification of Cochran's Q test for related observations with replication and unequal sample size. This test was used instead of a Signed Ranks test because of the large proportion of zeroes, ties, and the non-continuous nature of the data. There was a possible ( $\alpha$ =0.50) difference in the ability of thermally stressed and unstressed fathead minnows to avoid predators, as assessed by my test design.

The modification of Cochran's test was developed by Dr. Walter Pirie of the Virginia Polytechnic Institute and State University Statistics Department. The test statistic, an approximation of the  $\mathbf{X}^2$  test with one degree of freedom, is given below.

Table 2. Results of predation tests.

TEST NO.	NO. OF FISH PER RUN	NO. FISH EA	TEN PER RUN STRESS	TOTAL EATEN PER RUN (X <sub>i</sub> )
	30	7	6	13
I	30	5	7	12
	20	2	2	4
II	20	2	2	4
	16	0	2	2
III	16	4	4	8
	16	3	0	3
IV	16	2	3	5
	40	8	12	20
V	12	2	. 2	4
EXPE	L EATEN PER RIMENTAL GROUP or X.j.)	35	40	75

$$T = \frac{\left\{ \left( kC_{j} - \sum_{i} X_{i...} \right)^{2} \right.}{\left. \left\{ \frac{1}{n_{i}} X_{i...} \left( kn_{i} - X_{i...} \right) \right. \right\}}$$

Variables: k = number of treatments = 2  $n_i = number of observations per cell in block i$   $C_j = sum for column j$   $X_{i..} = sum for block i$ 

The value of T was calculated as 0.5522.

## Behavior

Averages of observations on activity, school size, and straying from tests II, III, IV, and V are given in tables 3, 4, and 5. I used Cox and Stuart's Test for Trends, from Conover (1971), to analyze data from average periodic observations from these tables. The signed ranks variation with comparison of end values was used for greater sensitivity to differences. The test was not applied to daily averages because the shortness in duration of the tests provided too few pairs of data for a robust test.

Activity. The average activity levels per day of the control vs. treatment fish appear to converge toward a common level of activity (Fig. 3). However, the stressed fish remained at a higher level of activity throughout the tests. The test period would have to be extended several days to statistically assess these trends.

Average activity levels throughout the average day (Fig. 4) showed differences between the stressed and control groups only during and immediately following morning and afternoon stressing periods. This observation agrees with that of Hoss et al. (1971) that increased

Table 3. Average activity values for each observation period.

				DAY				AVG. FOR
OBS. PER.	1	2	3	4	5	6	7	DAYS 1-6
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	1.30 1.30 1.50 1.50 1.10 1.40 1.10 1.30 1.10 1.40 1.50 1.30 2.00	1.30 1.40 1.00 1.30 1.00 1.40 1.60 1.10 1.30 1.30 1.40 1.50 1.30	1.30 1.50 1.10 1.00 1.10 1.10 1.30 1.30 1.30 1.40 1.50 1.40 2.00	1.00 1.40 1.50 1.40 1.30 1.00 1.00 1.20 1.40 1.60 2.00 1.30	1.30 1.40 1.30 1.30 1.30 1.30 1.30 1.30 1.30 1.3	1.00 1.70 1.10 1.50 1.00 1.50 1.30 1.30 1.30 2.00 1.30 2.00	1.00 1.50 1.30 1.40 1.40	1.20 1.45 1.27 1.33 1.13 1.35 1.30 1.03 1.20 1.28 1.32 1.40 1.75 1.32 1.70
AVG, DAY	1.32	1.28	1.29	1.34	1.36	1.40		
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	1.30 3.50 2.60 2.40 1.90 2.80 1.10 1.00 2.80 1.40 1.00 2.00 2.00	1.30 2.80 2.30 2.10 2.10 1.20 1.00 1.00 2.60 1.80 1.40 1.80 2.00 1.00	1.00 2.90 1.50 1.00 1.40 1.90 1.00 1.30 2.00 1.50 1.60 2.00 2.10 2.00	1.10 2.40 1.90 2.10 1.50 1.90 1.50 1.00 2.50 1.80 2.10 2.00 1.60	1.10 2.00 1.40 1.60 1.80 1.30 1.10 1.30 1.90 1.90 1.90 1.50	1.00 2.00 2.10 1.60 1.80 1.50 1.30 1.30 1.40 2.00 2.10 2.00	1.10 2.00 1.30 1.50 2.80	1.10 2.60 2.00 1.80 1.70 2.10 1.30 1.10 1.20 2.30 1.80 1.60 2.10 1.90
AVG.DAY	1.91	1.77	1.61	1.74	1.73	1.67		

Table 4. Average school size for each observation period.

					DAY				
OBS.PER. 1		. 1	2	3	4	5	6	7	AVG. FOR DAYS 1-6
	1. 2 3 4 5 6 7 8 9 10 11 12 13 14	7.25 6.18 3.50 6.50 8.75 6.58 7.60 10.90 9.40 6.63 6.10 7.10 4.00 8.45 15.00	5.00 5.85 3.38 6.75 5.38 7.24 2.30 4.88 9.00 6.00 9.25 7.80 9.50 5.44 6.50	5.19	13.63 10.70 9.13 8.63		8.38 15.00 9.63	PRED. TEST 63.6 9.63 9.63 9.63 9.63 9.63	6.17 5.06 3.71 7.45 8.90 7.95 8.58 11.87 10.09 7.76 7.85 8.65 10.33 7.89 10.90
AVG	. DAY	7.60	6.28	7.55	8.81	9.16	9.73		
	1 2 3 4 5 6 7 8 9 10 11 12 13 14	7.10 5.30 12.25 12.25 10.50 6.50 10.60 12.00 7.15 9.10 12.00 4.00 7.68 15.00	7.87 6.03 11.88 7.50 4.25 6.75 9.50 12.00 15.00 4.94 6.88 5.75 5.75 9.82 9.50	4.32 3.13 2.67 3.00 9.88 7.50 11.30 13.63 4.76 2.75 3.00 9.00 6.44 5.00	11.88 3.44 2.88 1.88 5.88 9.00 12.00 15.00 3.59 2.50 2.88 15.00 10.51	3.63 3.00 2.75 9.94 12.00 12.25 13.63 4.19 4.50 5.75 2.00 6.82 15.00	8.76 15.00	5.63 3.59 2.63 2.88 8.69	8.89 4.70 6.07 4.88 5.09 8.45 10.48 11.66 13.04 4.79 4.73 5.27 6.71 8.34 11.90
AVG	DAY	9 49	8.23	6.28	7.96	7.26	6.53		

Table 5. Average straying values for each observation period.

				DAY				
OBS. PER.	1	2	3	4	5	6	7	AVG. FOR DAYS 1-6
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	0.00 1.70 2.00 0.00 0.00 1.25 0.00 1.00 0.00 1.30 1.00 0.00 0.00	1.00 0.75 0.25 0.50 0.00 0.70 0.00 0.75 0.50 0.30 0.00  1.25 1.00	0.50 0.75 1.25 1.00 0.50 1.30 1.00  0.30 2.00 0.00 0.70  1.25 1.00	0.50 0.70 0.50 0.50 0.00 1.00 0.00 2.00  3.00 0.00 0.00	1.00 1.00 0.50 1.00 0.00 1.00 0.00 2.00 2.00 1.00 0.30 0.00 1.00	0.70 1.00 0.25 0.00 1.00 0.00 0.00 0.70 0.70 1.00	PRED, TEST .000 1.00 0.00 1.00 0.00 1.00	0.62 0.98 0.79 0.50 0.25 0.88 0.17 1.15 0.70 1.38 0.33 0.28 0.50 1.00
AVG.DAY	0.64	0.50	0.89	0.79	0.88	0.47		
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	0.00 2.75 3.00 1.00 0.00 2.75 1.50 1.00 0.00 1.30 1.50 0.00 0.00 2.30	0.00 1.30 3.00 1.70 0.30 2.25 0.00 0.00  1.25 1.00 0.00 1.00	0.70 0.00 2.50 0.00 0.30 1.00 0.50 1.00  0.75 1.00 1.75 0.00 0.70	0.00 1.25 1.30 0.75 0.30 1.30 0.00  1.00 1.00 1.25  1.70	0.30 0.00 1.00 0.75 1.25 1.00 0.00  1.25 1.00 0.50 0.00 2.00	1.00 1.00 1.25 0.25 0.00 2.50 0.00 0.30 2.00 1.00 0.25 1.00	0.00 2.00 0.50 0.50 0.25 1.00	0.33 1.05 2.18 0.74 0.36 1.80 0.33 0.40 0.15 1.26 1.08 0.79 0.20 1.57 0.50
AVG.DAY	1.22	0.99	0.73	0.90	0.70	0.88		

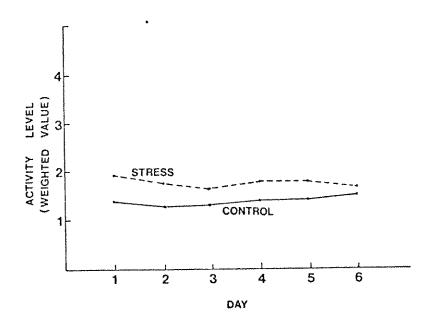


Figure 3. Average activity level per day for days one to six.

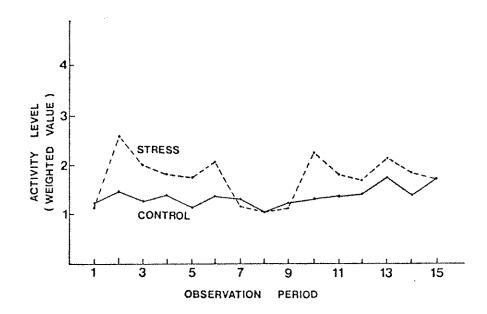


Figure 4. Average activity level per observation period.

temperature resulted on increased swimming rates. During the early morning (pre-stress), mid-day (the period of least disturbance), and the late day (post-stress) periods the activity levels of the two groups converged. The Cox and Stuart Test for Trends showed no trends (at  $\alpha=0.15$ ) of activity in either group.

School Size and Dispersal. The average daily school size of control vs. treated fish appeared to have opposite trends (Fig. 5), the control group becoming more dispersed over the week while the stressed group tended to form tighter schools. These apparent trends could not be adaquately tested by a trends test because of the limited data points.

The school size during an average day appeared to trend toward dispersal in the control group, whereas the stressed group tended to remain schooled (Fig. 6). Variability in both groups appeared to be high.

Analysis showed a significant trend of increasing school size of the control group at  $\approx 0.025$ , and a significant trend in the stressed group at  $\approx 0.20$ . The analysis agrees with visually apparent trends in Fig. 6.

Straying from the School. The average number of fish straying from the school per day in control and stressed groups exhibited possible convergance over time (Fig. 7). This observation suggests adjustment of schooling behavior to the stress. The number of strays at any one time appeared quite variable in both groups (Fig. 8). Cox and Stuart's Test for Trends showed that neither group exhibited definite (control  $\infty$ = 0.20, stressed  $\infty$ = 0.50) trends in the number of straying fish in the day.

Additional Behavioral Observations. Observations on additional

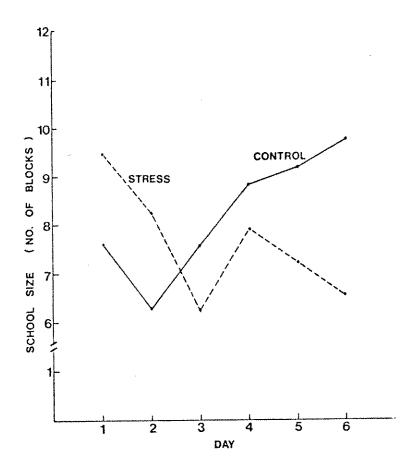


Figure 5. Average school size per day for days one to six.

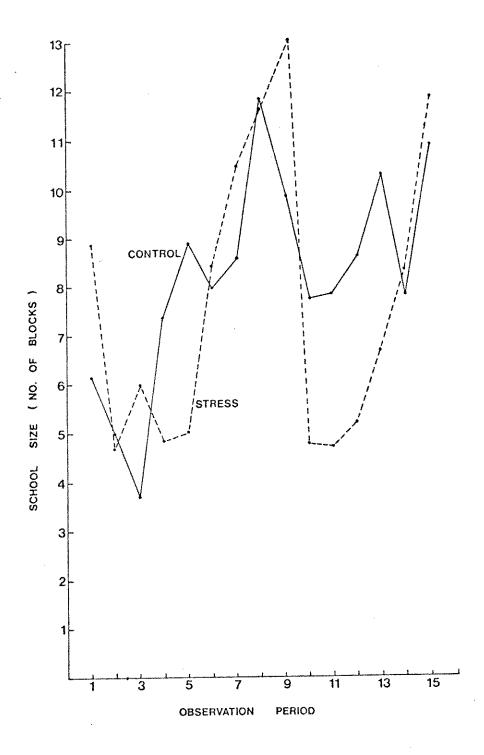


Figure 6. Average school size per observation period.

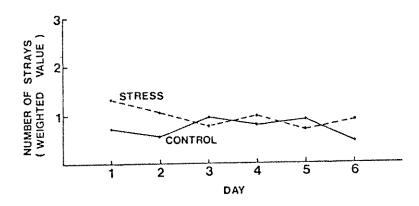


Figure 7. Average number of strays per day for days one to six.

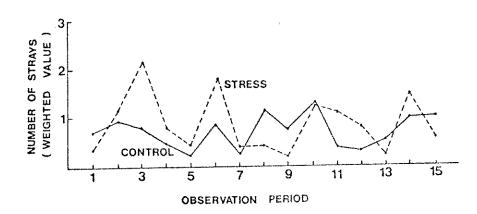


Figure 8. Average number of strays per observation period.

warrent further investigation. During each of the stressing periods, the school of stressed fish broke up and regrouped many times in rapid succession. The control group, in contrast, exhibited a much slower pulsation between schooling and dispersal. The periods of dispersal for the stressed group became longer as the water temperature approached 30C. The fish were, with few exceptions, completely dispersed when the water temperature reached 32C. The stressed fish often exhibited a rapid bottom feeding or pecking displacement type of behavior at the beginning of the stress periods. High swimming and ventillation rates, temporary loss of equilibrium and buoyancy control, and stupor were noted in various fish for short periods of time after water temperature reached 32C. Time of recovery varied among fish. Fish reacted most to the first few stress periods, with reactions to the stressing conditions becoming less intense as the week progressed.

In test IV, some social aggresiveness (chasing) was noted in the stressed fish during increasing temperature. I also noted apparent pairing of individuals in the bottom pecking actions. On day seven of test V, some of the control fish exhibited aggressiveness and the stressed group seemed to exhibit exceptionally high levels of activity. One possible explanation of the aggressiveness is the development of social hierarchies due to prolonged confinement.

The fright reaction exhibited by the stressed group when I disturbed the fish appeared to be less intense than that of the control group.

The predation tests indicate no significant effect of multiple exposures to maximum sublethal heat stress on the minnows ability to avoid predation by the bass. This apparent absence of any real difference is extremely interesting considering the work of Coutant (1973), which showed that thermal stress increased the susceptibility of juvenile salmon to predation. The absence of a similar result with fathead minnows leads me to hypothesize that temperature tolerant species (such as fathead minnows) perhaps recover more quickly than temperature intolerant species (such as salmonids) to sublethal thermal stress. If eurythermal fish are not as severely stressed by sublethal temperature shock as are stenothermal fish, physiological recovery may be more rapid. Since the salmonids in Coutants experiments were exposed to a single thermal shock while the minnows in this study were exposed to multiple shocks, experiments subjecting both types of fish to similar shocks would be required to substantiate this hypothesis.

The stressed groups exhibited high average activity levels and increasing tendencies to school while control groups maintained low average activity levels and showed increasing group dispersal.

Differences in activity and orientation within the tanks may have in part been due to differences in replication of water flow conditions in the behavior tanks. The control tank did not have a water exchange system because of economic limitations. In future tests both tanks should have water exchange systems controlled by the single thermoregulator.

There was no definite difference in number of fish straying from the stressed and control schools except possibly during actual stress periods. This factor does not appear to be of major importance under my test conditions as an indicator for effects of this type of stress on survival.

The lower intensity of fright reaction exhibited by the stressed group indicates that quantification of fright reaction may be valuable in further examination as an indicator of the effects of multiple thermal stress on survival.

It appears that more exact quantification of behavioral data would have been desirable. Variability in the subjective handling of observations may have affected results of the statistical analysis. I also believe tests with larger sample sizes and of longer duration may provide more reliable assessment of effects of the stress. In future tests, subdividing observation periods during temperature change and running predation tests during stress periods may also provide additional insight as to the effects of the stress on survival related behavior.

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